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Examiner	:	Susan Ungar
Applicants	:	Peter Brossart et al.
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For	:	Peptide for triggering an immune reaction against tumor cells

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF PETER BROSSART, M.D., UNDER 37 C.F.R. §1.132

Sir:

I, Peter Brossart, M.D., of University of Tübingen, Department of Hematology, and Oncology, D-72076 Tübingen, Germany, hereby declare:

THAT, my *curriculum vitae* is attached hereto as Exhibit A;

THAT, I am a named inventor on the above-referenced patent application;

THAT, through my years of research, I have kept up to date on the technical literature and maintained contact with experts in the field by participating in professional meetings and seminars, and by direct personal contact. As a result, I am familiar with the general level of skill of those working in the fields of immunology and clinical transplantation, and in particular the field of graft tolerance.

THAT, I have read and understood the specification and claims of the subject application and the respective Office and Advisory Actions;

AND, being thus duly qualified, do further declare:

The enclosed publication, Brossart *et al.* (The epithelial tumor antigen MUC1 is expressed in hematological malignancies and is recognized by MUC1-specific cytotoxic T, Cancer Res. 2001 Sep 15;61(18):6846-50; Exhibit B), of which I am co-author, in particular in Figures 4 and 5 as well as in the part titled "Materials and Methods" on page 6847 thereof clearly shows that both peptides as

currently claimed having the sequences STAPPVHNV and LLLLTVLTV from MUC1 have utility in that they specifically lyse primary cancer cells.

In detail, T-cells that specifically recognize the peptides in question were not only tested against cell lines that are representative for several tumors (cf. attached table 2), and with peptide-loaded antigen-presenting cells, (dendritic cells, cf. Figure 3), but also against primary cancer cells as obtained from four different cancer patients. The patients were suffering from Acute Myeloid Leukemia (AML), a common leukemia. Figure 4 clearly shows that not only a renal cancer cell line (A498), but also said primary cancer cells from four different cancer patients are lysed. Furthermore, the cell line SK-OV-3 was used as a HLA-A2-negative control.

Figure 5 furthermore supprts the results of Figure 4 in that it is shown that T-cells are specific for the peptides as offered, and that the amount of lysis is comparable to the control lysis of peptide-loaded croft-cells. In addition, there is no cross-reactivity regarding the peptide-recognition of the T-cells.

In summary, the above references provide strong evidence for a broad utility of the peptides of the invention in different cancer types, such as leukemia and renal cell cancer.

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or of any patent issuing thereon.

Further declarant sayeth naught.

Signed: _____
Peter Brossart, M.D.

Date: 02/21/06

Exhibit B

[CANCER RESEARCH 61; 6846–6850, September 15, 2001]

The Epithelial Tumor Antigen MUC1 Is Expressed in Hematological Malignancies and Is Recognized by MUC1-specific Cytotoxic T-Lymphocytes[1]

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ABSTRACT

The epithelial mucin MUC1 is overexpressed on the cell surface of many epithelial malignancies as well as on some B-cell lymphomas and multiple myelomas. Recently, we identified two HLA-A2-restricted T-cell epitopes derived from the MUC1 protein. To further extend the potential application of these peptides, we analyzed the expression of MUC1 on blast cells from patients with acute myelogenous leukemia (AML; $n = 43$) and several other hematological malignancies including acute lymphoblastic leukemia ($n = 24$), chronic lymphocytic leukemia ($n = 36$), hairy cell leukemia ($n = 9$), follicular lymphoma ($n = 7$), and multiple myeloma ($n = 12$). Using reverse transcription-PCR and MUC1-specific monoclonal antibodies, MUC1 expression was found in 67% of AML samples and 92% of myeloma samples. To analyze the presentation of MUC1 peptides by primary AML blasts, we induced MUC1-specific CTLs *in vitro* using peptide-pulsed dendritic cells from HLA-A2+ healthy donors as antigen-presenting cells. These CTLs efficiently lysed in an antigen-specific and HLA-A2-restricted manner not only target cells pulsed with the antigenic peptide but also tumor cell lines including multiple myeloma cells and primary AML blasts that constitutively expressed both MUC1 and HLA-A2. The specificity of the CTLs was confirmed in a cold target inhibition assay. Our data demonstrate that MUC1-derived peptides are tumor antigens in AML and several other hematological malignancies that could potentially be used for immunotherapeutic approaches.

INTRODUCTION

MUC1 is a highly glycosylated type I transmembrane glycoprotein that is abundantly overexpressed on the cell surface of many human adenocarcinomas like breast and ovarian cancers. Moreover, MUC1 expression has been demonstrated in multiple myeloma and some B-cell Non-Hodgkin lymphomas making MUC1 an attractive and broadly applicable target for immunotherapeutic strategies (1–9). Several recent reports (8–12) demonstrated that cytotoxic MHC-unrestricted T cells from ovarian, breast, pancreatic, and multiple myeloma tumors can recognize epitopes of the MUC1 protein core localized in the tandem repeat.

Recently (4), we identified two HLA-A2-binding peptides derived from the MUC1 protein. One of the peptides is derived from the tandem repeat region of the MUC1 protein, referred to as M1.1. The second peptide (referred to as M1.2) is localized within the signal sequence of MUC1. Using MUC1-peptide-pulsed DCs[3] as antigen-presenting cells, CTLs were generated that lysed tumors endogenously expressing MUC1 in an antigen-specific and HLA-A2-restricted fashion. More recently (13), we have shown that MUC1-specific CTLs could also be induced *in vivo* after vaccination of breast and ovarian cancer patients with peptide-pulsed DCs.

To extend the possible use of MUC1-derived T-cell epitopes in immunotherapeutic approaches, we screened the expression of MUC1 on normal hematopoietic cells (14) as well as on various hematological malignancies using monoclonal antibodies specific for the MUC1 tumor antigen. To prove the presentation of T-cell epitopes by the malignant cells, we induced MUC1-specific CTLs *in vitro* using peptide-pulsed DCs as antigen-presenting cells. We show here that the CTLs generated from several healthy donors by primary *in vitro* immunization elicited an antigen-specific and HLA-A2-restricted cytolytic activity against target cells endogenously expressing MUC1 including primary AML blasts and multiple myeloma cell lines, thus extending the number of malignancies expressing the MUC1 tumor-rejection antigen.

MATERIALS AND METHODS

Tumor Cell Lines. Tumor cell lines used in the experiments were grown in RP10 medium (RPMI 1640 supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine, 50 μM 2-mercaptoethanol, and antibiotics). The following tumors were used in experiments: MCF7 (MUC1+, HLA-A2+; purchased from the American Type Culture Collection), A498 (renal cell carcinoma, MUC1+, HLA-A2+), U266 (multiple myeloma, MUC1+, HLA-A2+), IM9 (multiple myeloma, MUC1+, HLA-A2+), Croft [EBV-immortalized B-cell line, kindly donated by O. J. Finn (Pittsburgh, PA), MUC1-, HLA-A2+], SK-OV-3 (ovarian cell line, MUC1+, HLA-A3; kindly provided by Dr. O. J. Finn, University of Pittsburgh School of Medicine). Blasts from patients with AML were grown in RP10 medium containing GM-CSF (Leukomax; Novartis, Basel, Switzerland; 100 ng/ml) for 2 days before they were used as target cells in a standard ^{51}Cr-labeled release assay.

Cell Isolation and Generation of DCs from Adherent PBMNCs. Generation of DCs from peripheral blood monocytes was performed as described previously (4, 15, 16). In brief, PBMNCs were isolated by Ficoll/Paque (Life Technologies, Inc.) density gradient centrifugation of heparinized blood obtained from buffy coat preparations of healthy volunteers from the blood bank of the University of Tübingen. Cells were seeded (1×10^7 cells/3 ml/well) into 6-well plates (Costar, Cambridge, MA) in RP10 media (RPMI 1640 supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine, 50 μM 2-mercaptoethanol, and antibiotics). After 2 h of incubation at 37°C, nonadherent cells were removed, and the adherent blood monocytes were cultured in RP10 medium supplemented with the following cytokines: human recombinant GM-CSF (Leukomax; Novartis; 100 ng/ml), IL-4 (Genzyme; 1000 IU/ml), and TNF-α (Genzyme; 10 ng/ml). The phenotype of DCs was analyzed by flow cytometry after 7 days of culture. Isolation of CD14+ monocytes, CD15+ granulocytes, and CD34+ peripheral blood progenitor cells was performed using MACS technology, as recommended by the manufacturer. The purity of the cells was >90%.

Immunostaining. Cell staining was performed using FITC- or PE-conjugated mouse monoclonal antibodies against CD86, CD40 (PharMingen, Hamburg, Germany), CD80, HLA-DR, CD54, CD14 (Becton Dickinson, Heidelberg, Germany), CD83 (Coulter-Immunotech, Hamburg, Germany), and CD1a (OKT6; Ortho Diagnostic Systems). Appropriate mouse IgG isotypes were used as controls (Becton Dickinson). The level of HLA-A2 expression was analyzed using a purified monoclonal antibody specific for HLA-A2 (BB7.2; data not shown). The MUC1 expression was determined using the monoclonal antibodies BM-2, BM-7 (Ref. 17; kindly provided by Dr. Sepp Kaul, University of Heidelberg, Heidelberg, Germany), and HMFG-1 (Ref. 4; IgG1; No-

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[3] The abbreviations used are: DC, dendritic cell; AML, acute myeloid leukemia; GM-CSF, granulocyte macrophage colony-stimulating factor; IL, interleukin; PBMNC, peripheral blood mononuclear cell; TNF, tumor necrosis factor; RT-PCR, reverse transcription-PCR; CLL, chronic lymphocytic leukemia.

Table 1 *Expression of MUC-1 (BM-2) on primary malignant cells from patients with various hematological malignancies*

		Percentage of positive samples	MFI* Mean ± SD (range)
B-CLL	$n = 36$	19% (7/36)	5.6 (3.6–9.1)
T-CLL	$n = 2$	100% (2/2)	48.4 ± 38.3 (21.3–75.5)
HCL	$n = 9$	22% (2/9)	30.6 ± 3.5 (28.1–33.0)
FL	$n = 7$	43% (3/7)	3.6 (3.0–4.7)
MCL	$n = 8$	13% (1/8)	4.1
MZL	$n = 4$	25% (1/4)	4.6
Myeloma	$n = 12$	92% (11/12)	134.4 (5.3–657.2)
T-ALL	$n = 6$	0% (0/6)	
Common-ALL	$n = 24$	33% (8/24)	17.8 ± 9.9 (4.3–32.0)
AML	$n = 43$	67% (29/43)	21.3 ± 19.7 (4.2–78.7)
AML-M1	$n = 6$	50% (3/6)	8.7 ± 4.5 (5.0–13.8)
AML-M2	$n = 4$	50% (2/4)	9.8 ± 1.5 (8.7–10.8)
AML-M4	$n = 11$	64% (7/11)	22.7 ± 19.3 (5.3–56.1)
AML-M5	$n = 15$	87% (13/15)	23.7 ± 18.2 (4.2–72.6)
AML unclassified	$n = 7$	57% (4/7)	26.6 ± 34.9 (4.8–78.7)
CML-BCmy	$n = 9$	56% (5/9)	13.3 ± 4.0 (7.2–17.9)
CML-BCly	$n = 2$	0% (0/2)	

* MFI, median fluorescence intensity. MFI levels <3 were considered negative, levels >3 were considered to be positive; HCL, hairy cell leukemia; FL, follicular lymphoma; MCL, mantle cell lymphoma; MZL, marginal cell lymphoma; c-ALL, common acute lymphoblastic leukemia; CML-BCmy, chronic myelogenous leukemia in myeloid blast crisis; CML-BCly, chronic myelogenous leukemia in lymphoid blast crisis.

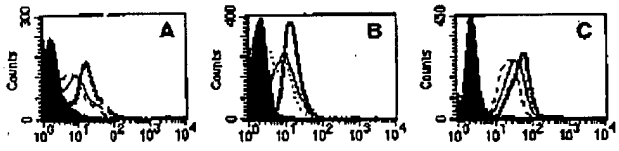

Fig. 1. Flow cytometric analysis of MUC1 expression on AML blasts obtained from three different patients. The MUC1 expression was determined using unlabeled antibodies BM2 (*bold line*), BM7 (*thin solid line*), and HMFG-1 (*dotted line*) by staining with FITC-conjugated goat antimouse antibody. *Filled histograms* represent isotype-matched controls.

Novocastra Laboratories, Newcastle, United Kingdom), followed by FITC-conjugated goat antimouse antibody (Becton Dickinson). The samples were analyzed on a FACScan Calibur (Becton Dickinson).

RT-PCR. RT-PCR was performed with some modifications as described recently (14). Total RNA was isolated from cell lysates using Qiagen RNeasy "Mini" anion-exchange spin columns (Qiagen, Hilden, Germany) according to the instructions of the manufacturer. For standardization of the various PCR experiments, 1.5 μg, 2.5 μg, or 800 ng of total RNA, depending on the different amounts isolated, were subjected to a 20-μl cDNA synthesis reaction (SuperScript First-Strand Synthesis System for RT-PCR; Life Technologies, Inc., Karlsruhe, Germany). Oligodeoxythymidylate was used as primer. cDNA (2 μl) were used for PCR amplification. To control the integrity of the RNA and the efficiency of the cDNA synthesis, 1 μl of cDNA was amplified by an intron-spanning primer pair for the *β2-microglobulin* gene. The PCR temperature profiles were as follows: 5-min pretreatment at 94°C and 22 or 25 cycles at 94°C for 15 s, annealing at 55°C for 30 s and 72°C for 30 s for the *β2-microglobulin* gene. For the *MUC-1* gene, 5-min pretreatment at 94°C, 35 cycles at 94°C for 15 s, and annealing at 60°C for 30 s and 72°C for 30 s were applied. Primer sequences were deduced from published cDNA sequences: β2-microglobulin, 5′-GGGTTTCATCCATCCGACAT-3′ and 5′-GATGCTGCTTACATGTCTCGA-3′; and MUC1, 5′-CGTCGTGGACATTGATGGTACC-3′ and 5′-GGTACCTCCTCTCACCTCCTCCAA-3′. Ten μl of the RT-PCR reactions were electrophoresed through a 3% agarose gel and stained with ethidium bromide for visualization under UV light.

Induction of Antigen-specific CTL Response Using HLA-A2-restricted Synthetic Peptides. The MUC1-derived peptides M1.1 (amino acids 950–958, STAPPVHNV from the tandem repeat domain) and M1.2 (amino acids 12–20, LLLLTVLTV from the leader sequence) were synthesized using standard F-moc chemistry on a peptide synthesizer (432A; Applied Biosystems, Weiterstadt, Germany) and analyzed by reversed-phase high-performance liquid chromatography and mass spectrometry (4). For CTL induction, 5×10^5 DCs were pulsed with 50 μg/ml synthetic peptide for 2 h, washed, and incubated with 2.5×10^6 autologous PBMNCs in RP10 medium. After 7 days of culture, cells were restimulated with autologous peptide-pulsed PBMNCs, and 1 ng/ml human recombinant IL-2 (Genzyme) was added on days 1, 3, and 5. The cytolytic activity of induced CTL was analyzed on day 5 after the last restimulation in a standard ^{51}Cr-labeled release assay (4, 16).

CTL Assay. The standard ^{51}Cr-labeled release assay was performed as described (4, 16). Target cells were pulsed with 50 μg/ml peptide for 2 h and labeled with [^{51}Cr]sodium chromate in RP10 for 1 h at 37°C. Cells (10^4) were transferred to a well of a round-bottomed 96-well plate. Varying numbers of CTLs were added to give a final volume of 200 μl and incubated for 4 h at 37°C. At the end of the assay, supernatants (50 μl/well) were harvested and counted in a β-plate counter. The percentage of specific lysis was calculated as: 100 × (experimental release − spontaneous release/maximal release − spontaneous release). Spontaneous and maximal releases were determined in the presence of either medium or 1% Triton X-100, respectively.

Antigen specificity of tumor cell lysis was further determined in a cold target inhibition assay (4) by analyzing the capacity of peptide-pulsed unlabeled Croft cells to block lysis of tumor cells at a ratio of 20:1 (inhibitor:target ratio).

RESULTS

Expression of MUC1 Tumor Antigen on AML Blasts. The expression of MUC1 on malignant hematopoetic cells was determined using the MUC1-specific monoclonal antibody BM-2 (14, 17). As demonstrated in Table 1, MUC1 expression could be detected in 92% of the samples from multiple myeloma patients and in 67% of the blast samples from patients with AML. Interestingly, the frequency and the level of MUC1 expression were higher on AML FAB M4 and M5 blasts as compared with the FAB M1 and M2 subtypes (Table 1). An example of MUC1 expression on AML blasts from three patients is presented in Fig. 1 where three different MUC1-specific antibodies were used. The protein expression results obtained by flow cytometry were further confirmed using RT-PCR and MUC1-specific primers (Fig. 2*A*). Interestingly, as demonstrated in Fig. 2*B*, we could not detect any MUC1 transcripts in other myeloid cells purified from peripheral blood like CD14+ (monocytes) and CD15+ (granulo-

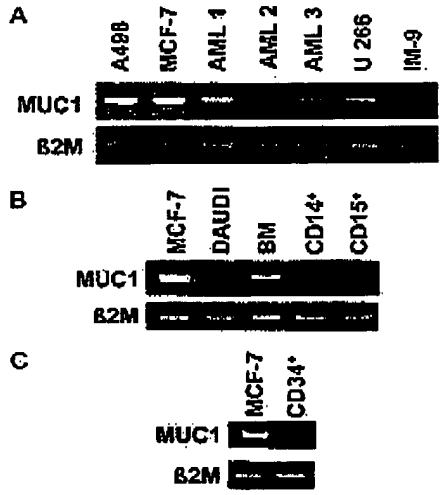

Fig. 2. MUC1 mRNA expression in purified peripheral blood and bone marrow cells, human tumor cell lines, and primary AML blasts analyzed by RT-PCR; 1.5 μg (*A*), 2.5 μg (*B*), and 800 ng (*C*) of total RNA were subjected to cDNA synthesis as described in "Materials and Methods." Thirty-five rounds of PCR amplification for MUC1 cDNA and 22 (*A* and *B*) and 25 cycles (*C*) for β2-microglobulin cDNA were performed. PCR products were run on a 3% agarose gel and visualized by ethidium bromide staining. MUC1-positive cell lines MCF7, U266, A498, and MUC1-negative IM9 cells were used as controls.

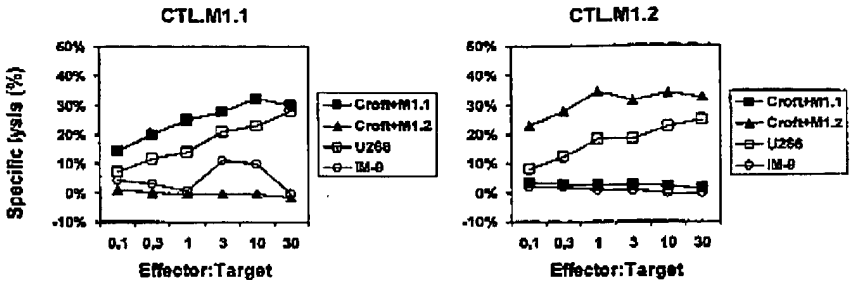

Fig. 3. Induction of MUC1-specific CTL responses by peptide-pulsed mature DCs. Adherent PBMNCs were grown for 7 days in RP10 medium supplemented with GM-CSF, IL-4, and TNF-α. DCs pulsed with the synthetic peptides derived from the MUC1 protein (M1.1 and M1.2) were used to induce a CTL response *in vitro*. Cytotoxic activity of induced CTL (CTL.M1.1 and CTL.M1.2) was determined in a standard ^{51}Cr-labeled release assay using the multiple myeloma cell lines U266 (MUC1+, HLA-A2+) and IM9 (MUC1−, HLA-A2+), and the EBV-immortalized B-cell line Croft (MUC1−, HLA-A2+) as targets. Croft cells were pulsed for 2 h with 50 μg of the M1.1 (■) or M1.2 peptide (▲).

Table 2 *Lysis of human tumor cell lines by MUC1-specific CTL*

Tumor cell lines[a]	HLA-A2 expression	MUC1 expression	CTL.M1.1 Percentage of specific lysis at E:T 3	1	0.3	CTL.M1.2 Percentage of specific lysis at E:T 3	1	0.3
A-498	+	+	28	22	14	22	14	4
U266	+	+	19	15	7	16	26	3
SK-OV-3	−	+	−1	2	−2	−2	−3	−2
IM-9	+	−	−15	−4	−2	−14	−13	−12

[a] The cytotoxic activity of CTL.M1.1 and CTL.M1.2 against human tumor cell lines was analyzed in a standard ^{51}Cr-labeled release assay. The HLA-A2 and MUC1 expression was analyzed using specific monoclonal antibodies. Data are presented from one representative experiment.

cytes) cells. Analysis of bone marrow mononuclear cells revealed MUC1 mRNA expression, as expected, according to our previous observation where we could show that a small fraction of bone marrow mononuclear cells representing the erythroid progenitor cell compartment (erythroblasts and normoblasts) express the MUC1 protein (see Ref. 14). In addition, we observed that some CD34^{low} cells express MUC1 on the cell surface, whereas the more primitive CD34^{bright}CD90^{+} progenitor/stem cell population was MUC1-negative (see Ref. 14). Similarly, as shown in Fig. 2*C*, we detected only a weak MUC1 mRNA signal in purified CD34+ cells by RT-PCR. These results suggest that the aberrant MUC1 expression on AML blasts is presumably a result of oncogenic transformation.

Induction of MUC1-specific CTLs Using Peptide-pulsed DCs. To analyze the expression of MUC1-derived T-cell epitopes by primary AML cells, we induced MUC1-specific CTLs *in vitro* using peptide-pulsed DCs as antigen-presenting cells, as described previously (4). DCs derived from adherent PBMNCs of healthy donors were generated in RP10 medium supplemented with GM-CSF, IL-4, and TNF-α.

The two recently described MUC1-derived peptides M1.1 (amino acids 950–958) and M1.2 (amino acids 12–20) were used for CTL induction *in vitro* (4). As shown in Fig. 3, CTL lines CTL.M1.1 and CTL.M1.2 obtained after 2 weekly restimulations demonstrated peptide-specific killing. T-cells only recognized Croft cells coated with the cognate MUC1 peptide, whereas they did not lyse cells pulsed with an irrelevant peptide.

We further investigated the ability of CTL.M1.1 and CTL.M1.2 to lyse endogenously MUC1-expressing tumor cells. The MUC1-positive, HLA-A2-expressing cell lines U266 (multiple myeloma) and A498 (renal cell carcinoma) were used as target cells in a standard ^{51}Cr-labeled release assay. As demonstrated in Fig. 3 and Table 2, both CTL lines were able to efficiently lyse U266 and A498 cells (both HLA-A2+/MUC1+). There was no lysis of the ovarian cancer cells SK-OV-3 (MUC1+/HLA-A3+), IM9 cells (multiple myeloma; HLA-A2+, MUC1−), or Croft cells (HLA-A2+, MUC1−). These results demonstrate that the presentation of MUC1 epitopes in context of HLA-A2 molecules on the target cells is necessary for the efficient lysis of target cells and confirm the antigen specificity and MHC restriction of the CTL. Furthermore, these data show that the MUC1 peptides can be presented in an HLA-restricted manner by multiple myeloma cells on the cell surface.

Lysis of Primary AML Blasts by MUC1-specific CTLs. Flow cytometric analysis of different tumor cell types revealed MUC1 expression on several malignant hematopoetic cells including blasts obtained from patients with AML (Fig. 1; Table 1). Therefore, we analyzed the presentation of MUC1-derived peptides by these primary AML blasts and used them as targets in a standard ^{51}Cr-labeled release assay. As shown in Fig. 4, CTL.M1.1 and CTL.M1.2 did lyse primary MUC1-expressing AML blasts obtained from HLA-A2-positive patients, suggesting that MUC1 peptides are presented by these leukemias. In contrast, there was no lysis of control cell lines.

The antigen specificity and MHC restriction mediated by the *in vitro* induced CTL lines was further confirmed in a cold target inhibition assay (Fig. 5). The lysis of the AML cells (*AMLSch*) could be blocked by addition of Croft cells pulsed with the cognate peptide, whereas cells pulsed with an irrelevant peptide showed no effect.

DISCUSSION

Recently, several attempts have been made to define possible leukemia-specific CTL epitopes. Fusion proteins such as BCR-ABL

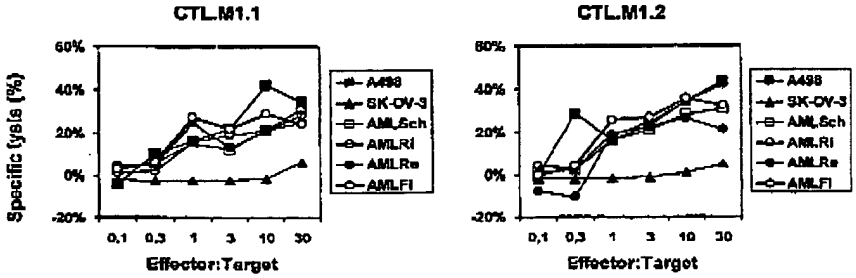

Fig. 4. Lysis of AML blasts endogenously expressing MUC1 by CTL.M1.1 and CTL.M1.2. Human renal carcinoma cell line A498 (HLA-A2+/MUC1+), ovarian cancer cell line SK-OV-3 (HLA-A2−/MUC1+), and primary MUC1-expressing allogeneic AML blasts from four different HLA-A2-positive patients were used as targets in a standard ^{51}Cr-labeled release assay.

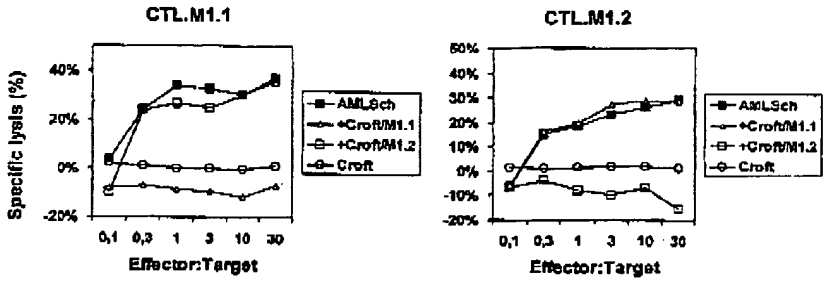

Fig. 5. Antigen-specific lysis of human AML blasts by MUC1-reactive CTL.M1.1 and CTL.M1.2. Cold target inhibition assay. Human AML blasts (AMLSch, HLA-A2+, and MUC1+) were used as targets in a standard ^{51}Cr-labeled release assay. The antigen specificity of the CTL lines was tested in the presence of unlabeled cold targets, *i.e.*, Croft cells, coated with the cognate or an irrelevant peptide at an inhibitor:target ratio of 20:1.

in chronic myelogenous leukemia, ETV6-AML1 in pre-B acute lymphoblastic leukemia, PML-RARα in acute promyelocytic leukemia, and DEK-CAN in AML resulting from chromosomal translocations are potential targets because they are expressed only in malignant cells (18). Furthermore, they represent novel antigens and, in contrast to self-proteins, are not associated with the phenomenon of tolerance.

An alternative strategy to identify tumor- or leukemia-specific T-cell epitopes is the use of synthetic antigenic peptides derived from proteins that are preferentially expressed or overexpressed in malignant cells like MAGE, HER-2/neu, p53, or MUC1. However, thus far, only peptides from proteinase 3 and WT1 proteins have been demonstrated to elicit antigen-specific lysis of leukemic cells by cytotoxic T cells (19, 20).

We now demonstrate that the epithelial mucin, MUC1, is a novel tumor antigen in AML that is recognized by MUC1 peptide-specific CTLs. Recently, we identified two HLA-A2-binding peptides, M1.1 and M1.2, derived from the MUC1 protein. These peptide epitopes were expressed on various epithelial malignancies and were recognized by MUC1-specific CTLs (4). To extend the possible use of these peptides in vaccination therapies, we analyzed the expression of the MUC1 tumor antigen on various hematopoietic malignancies including AML, multiple myeloma, follicular lymphoma, hairy cell leukemia, and CLL using MUC1-specific monoclonal antibodies. We found that MUC1 is expressed in 92% of samples from patients with multiple myeloma and about 67% of blast samples obtained from patients with AML, especially on AML FAB M4 and M5 subtypes. In addition, MUC1 protein expression was also observed on blasts from chronic myelogenous leukemia patients with myeloid blast crisis. Finally, MUC1 expression was detected on some follicular lymphomas, CLLs, and hairy cell leukemia samples (see Table 1).

To analyze whether MUC1-derived T-cell epitopes are presented by AML cells endogenously expressing MUC1, we induced MUC1 peptide-specific CTLs *in vitro* and used these CTLs to determine the presentation of MUC1 peptides on primary AML blasts. DCs generated from normal HLA-A2+ peripheral blood monocytes in the presence of GM-CSF, IL-4, and TNF-α were pulsed with the MUC1 peptides M1.1 and M1.2 and used as antigen-presenting cells for CTL priming. The MUC1 peptide-specific CTL lines CTL M1.1 and CTL M1.2 were able to recognize not only target cells pulsed with the antigenic peptide but also primary leukemic blasts and tumor cells endogenously expressing the MUC1 protein in an HLA-A2-restricted manner, including the multiple myeloma cell line U266. These results are complementary to reports published previously (11, 12) demonstrating that multiple myeloma cells can be lysed by MHC unrestricted MUC1-specific CTLs. In our study, both M1.1- and M1.2-specific CTLs efficiently lysed primary allogeneic AML blasts from HLA-A2-positive patients. The antigen specificity of this cytotoxic effect was confirmed in a cold target inhibition assay.

Although not experimentally demonstrated here, it is likely that the MUC1-derived T-cell epitopes M1.1 and M1.2 might also be expressed by some B-CLL cells, hairy cells, and follicular lymphoma cells. Interestingly, MUC1 has been shown recently (21) to be rearranged and amplified in B-cell lymphomas by the t(1;14) translocation. The authors have shown that up to 16% of B-cell lymphomas show a molecular perturbation of the MUC1 region that can potentially lead to its deregulated overexpression. According to our results, the aberrant expression of MUC1 on AML blasts could also represent an oncogenic transformation, particularly because we could not observe MUC1 expression on the normal CD14+, CD15+, and the CD34^{bright} cell populations (14).

There is now growing evidence that *in vivo* application of DC-presenting tumor-associated antigens or adoptive transfer of tumor-reactive CTLs generated *ex vivo* can induce antitumor immunity in patients with malignant diseases (13, 22–26). In a Phase I study using DCs pulsed with HLA-A2-binding peptides derived from Her-2/neu or MUC1 tumor antigens, we were recently able to induce peptide-specific CTLs in patients with metastatic breast and ovarian cancers *in vivo* without any side effects or autoimmune reactions, especially no induction of anemia, demonstrating that MUC1 peptides can be safely and efficiently applied in clinical studies (13). In addition, although MUC1 is expressed on normal cells in the gastrointestinal tract and several other tissues including breast and kidney, we did not observe any side effects during DC vaccinations. This might be related to the lower affinity of the induced MUC1-specific T cells (4) or because of the higher presentation of MUC1-derived peptides by tumor cells.

In conclusion, our results extend the list of malignancies including AML and multiple myeloma that present MUC1-derived T cell epitopes, which increases the possible clinical application of MUC1-derived peptides in vaccination studies.

REFERENCES

1. Gendler, S., Taylor-Papadimitriou, J., Duhig, T., Rothbard, J., and Burchell, J. A highly immunogenic region of a human polymorphic epithelial mucin expressed by carcinomas is made up of tandem repeats. J. Biol. Chem., *263:* 12820–12823, 1988.
2. Siddiqui, J., Abe, M., Hayes, D., Shani, E., Yunis, E., and Kufe, D. Isolation and sequencing of a cDNA coding for the human DF3 breast carcinoma-associated antigen. Proc. Natl. Acad. Sci. USA, *85:* 2320–2323, 1988.
3. Girling, A., Bartkova, J., Burchell, J., Gendler, S., Gillett, C., and Taylor-Papadimitriou, J. A core protein epitope of the polymorphic epithelial mucin detected by the monoclonal antibody SM-3 is selectively exposed in a range of primary carcinomas. Int. J. Cancer, *43:* 1072–1076, 1989.
4. Brossart, P., Heinrich, K. S., Stuhler, G., Behnke, L., Reichardt, V. L., Stevanovic, S., Muhm, A., Rammensee, H. G., Kanz, L., and Brugger, W. Identification of HLA-A2-restricted T-cell epitopes derived from the MUC1 tumor antigen for broadly applicable vaccine therapies. Blood, *93:* 4309–4317, 1999.
5. Duperray, C., Klein, B., Durie, B. G., Zhang, X., Jourdan, M., Ponculet, P., Favier, F., Vincent, C., Brochier, J., and Lenoir, G. Phenotypic analysis of human myeloma cell lines. Blood, *73:* 566–572, 1989.
6. Mark, A. S., and Mangkornkanok, M. B-cell lymphoma marking only with anti-epithelial membrane antigen. Cancer (Phila.), *63:* 2152–2155, 1989.
7. Delsol, G., Al Saati, T., Gatter, K. C., Gerdes, J., Schwarting, R., Caveriviere, P., Rigal-Huguet, F., Robert, A., Stein, H., and Mason, D. Y. Coexpression of epithelial membrane antigen (EMA), Ki-1, and interleukin-2 receptor by anaplastic large cell lymphomas. Diagnostic value in so-called malignant histiocytosis. Am. J. Pathol., *130:* 59–70, 1988.

8. Apostolopoulos, V., and McKenzie, I. F. Cellular mucins: targets for immunotherapy. Crit. Rev. Immunol., *14:* 293–309, 1994.
9. Finn, O. J., Jerome, K. R., Henderson, R. A., Pecher, G., Domenech, N., Magarian-Blander, J., and Barratt-Boyes, S. M. MUC-1 epithelial tumor mucin-based immunity and cancer vaccines. Immunol. Rev., *145:* 61–89, 1995.
10. Barnd, D. ..., Lan, M. S., Metzgar, R. S., and Finn, O. J. Specific, major histocompatibility complex-unrestricted recognition of tumor-associated mucins by human cytotoxic ' cells. Proc. Natl. Acad. Sci. USA, *86:* 7159–7163, 1989.
11. Takahashi, T., Makiguchi, Y., Hinoda, Y., Kakiuchi, H., Nakagawa, N., Imai, K., and Yachi, A. Expression of MUC1 on myeloma cells and induction of HLA-unrestricted CTL against MUC1 from a multiple myeloma patient. J. Immunol., *153:* 2102–2109, 1994.
12. Noto, H., Takahashi, T., Makiguchi, Y., Hayashi, T., Hinoda, Y., and Imai, K. Cytotoxic T lymphocytes derived from bone marrow mononuclear cells of multiple myeloma patients recognize an underglycosylated form of MUC1 mucin. Int. Immunol., *9:* 79 –798, 1997.
13. Brossart, P., Wirths, S., Stuhler, G., Reichardt, V. L., Kanz, L., and Brugger, W. Induction of cytotoxic T-lymphocyte responses *in vivo* after vaccinations with peptide-pulsed dendritic cells. Blood, *96:* 3102–3108, 2000.
14. Brugger, W., Buhring, H. J., Grunebach, F., Vogel, W., Kanz, L., Muller, R., Brummendorf, T. H., Ziegler, B. L., Rappold, I., Brossart, P., Scheding, S., and Kanz, L. Expression of MUC-1 epitopes on normal bone marrow: implications for the detection of micrometastatic tumor cells. J. Clin. Oncol., *17:* 1535–1544, 1999.
15. Brossart, P., Grunebach, F., Stuhler, G., Reichardt, V. L., Mohle, R., Kanz, L., and Brugger, W. Generation of functional human dendritic cells from adherent peripheral blood monocytes by CD40 ligation in the absence of granulocyte-macrophage colony-stimulating factor. Blood, *92:* 4238–4247, 1998.
16. Brossart, P., Stuhler, G., Flad, T., Stevanovic, S., Rammensee, H. G., Kanz, L., and Brugger, W. Her-2/neu-derived peptides are tumor-associated antigens expressed by human renal cell and colon carcinoma lines and are recognized by *in vitro* induced specific cytotoxic T lymphocytes. Cancer Res., *58:* 732–736, 1998.
17. Brummendorf, T. H., Kaul, S., Schuhmacher, J., Baum, R. P., Matys, R., Klivenyi, G., Adams, S., and Bastert, G. Immunoscintigraphy of human mammary carcinoma xenografts using monoclonal antibodies 12H12 and BM-2 labeled with 99mTc and radioiodine. Cancer Res., *54:* 4162–4168, 1994.
18. Jurcic, J. G., Cathcart, K., Pinilla-Ibarz, J., and Scheinberg, D. A. Advances in immunotherapy of hematologic malignancies: cellular and humoral approaches. Curr. Opin. Hematol., *7:* 247–254, 2000.
19. Molldrem, J. J., Clave, E., Jiang, Y. Z., Mavroudis, D., Raptis, A., Hensel, N., Agarwala, V., and Barrett, A. J. Cytotoxic T lymphocytes specific for a nonpolymorphic proteinase 3 peptide preferentially inhibit chronic myeloid leukemia colony-forming units. Blood, *90:* 2529–2534, 1997.
20. Ohminami, H., Yasukawa, M., and Fujita, S. HLA class I-restricted lysis of leukemia cells by a CD8(+) cytotoxic T-lymphocyte clone specific for WT1 peptide. Blood, *95:* 286–293, 2000.
21. Dyomin, V. G., Palanisamy, N., Lloyd, K. O., Dyomina, K., Jhanwar, S. C., Houldsworth, J., and Chaganti, R. S. MUC1 is activated in a B-cell lymphoma by the t(1;14)(q21;q32) translocation and is rearranged and amplified in B-cell lymphoma subsets. Blood, *95:* 2666–2671, 2000.
22. Timmerman, J. M., and Levy, R. Dendritic cell vaccines for cancer immunotherapy. Annu. Rev. Med., *50:* 507–529, 1999.
23. Nestle, F. O., Alijagic, S., Gilliet, M., Sun, Y., Grabbe, S., Dummer, R., Burg, G., and Schadendorf, D. Vaccination of melanoma patients with pep. Nat. Med., *4:* 328–332, 1998.
24. Hsu, F. J., Benike, C., Fagnoni, F., Liles, T. M., Czerwinski, D., Taidi, B., Engleman, E. G., and Levy, R. Vaccination of patients with B-cell lymphoma using autologous antigen-pulsed dendritic cells. Nat. Med., *2:* 52–58, 1996.
25. Thurner, B., Haendle, I., Roder, C., Dieckmann, D., Keikavoussi, P., Jonuleit, H., Bender, A., Maczek, C., Schreiner, D., von den, D. P., Brocker, E. B., Steinman, R. M., Enk, A., Kampgen, E., and Schuler, G. Vaccination with mage-3A1 peptide-pulsed mature, monocyte-derived dendritic cells expands specific cytotoxic T cells and induces regression of some metastases in advanced stage IV melanoma. J. Exp. Med., *190:* 1669–1678, 1999.
26. Kugler, A., Stuhler, G., Walden, P., Zoller, G., Zobywalski, A., Brossart, P., Trefzer, U., Ullrich, S., Muller, C. A., Becker, V., Gross, A. J., Hemmerlein, B., Kanz, L., Muller, G. A., and Ringert, R. H. Regression of human metastatic renal cell carcinoma after vaccination with tumor cell-dendritic cell hybrids (see comments). Nat. Med., *6:* 332–336, 2000.

Table 2

Cell line	HLA-A2-Expression	MUC1-Expression	Tumor
MCF7	+	+	breast cancer
A498	+	+	renal cell carcinoma
U266	+	+	multiple myeloma
IM9	+	-	multiple myeloma
Croft	+	-	EBV-immortalized B-cell line
SK-OV-3	-	+	ovarian cell line

Exhibit A

Curriculum vitae

(PD Dr. med. Peter Brossart, born 24.01.1963)

Business address

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E-Mail: peter.brossart@med.uni-tuebingen.de

School-leaving examination

5/ 984 — Friedrich-List-Gymnasium Reutlingen

Course of studies

10/1984 – 5/1991 — Studying human medicine, University Tübingen

6/ 991 — Receiving the doctoral degree in medicine; University Tübingen, Thesis: "Influence of insulin, proinsulin, IGF-I and protirelin on the growth and the iodide-uptake of cultivated pig erythrocytes".
Degree: very good

Professional experience

6/ 991 – 8/1992 — Resident at the University Medical Clinic Freiburg, Department of Hematology and Oncology
Main research: immunomodulatory effects of IL-2 in vivo (BMFT supported project), immunotherapy with recombinant cytokines (IL-2, IL-1)

9/ 992 – 1/1995 — Research associate at the University Medical Clinic Heidelberg, Department of Hematology, Oncology and Rheumatology
Main research: immunotherapy of patients having malignant melanoma (cytokine, peptide), detection of circulating tumor cells in the periphery blood using RT-PCR (founded by the German cancer aid)

since 1/1997 — Research associate at the University Medical Clinic Tübingen, Department of Hematology, Oncology, Immunology and Rheumatology
Main research: development of immunotherapies with antigen-loaded dendritic cells (DC), development and function of DC, interaction between DC and T-Lymphocytes, identification of new tumor-antigens (sine 1997 Member of the SFB 510, founded by the German cancer aid as well as by the *fortune*-program of the University Tübingen)

since 12/1999 — Internist

since 6/2000	Representative of the Medical Clinic at the interdisciplinary conference for dermal tumor
13.02.2001	Habilitation in Internal Medicine
since 2/2001	Senior physician, University Medical Clinic Tübingen
since 1/2001	Head of the department of experimental Immunotherapies

Temporary employment abroad

2/1995 – 1/1997	Research fellowship of the German cancer aid at the Howard-Hughes-Medical-Institute, Department of immunology, University of Washington, Seattle, USA (Dr. M. J. Bevan)

Awards

6/1993	Poster Award, Third International Symposium on Cytokines in Hematology, Oncology, and Immunology in Hannover, June 23, 1993
10/1993	Poster Award, conferred by the German Society for Hematology and Oncology
10/1998	Arthur-Pappenheim Award for Hematology and hematological Oncology, conferred by the German Society for Hematology and Oncology
1[illegible]/2000	Best Abstract Award, conferred by the German Society for Hematology and Oncology
12/2000	Award of the American Society of Hematology

Lebenslauf (PD Dr. med. Peter Brossart, geb. 24.01.1963)

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Reifeprüfung

5/1984 Friedrich-List-Gymnasium Reutlingen

Studium

10/1984 - 5/1991 Studium der Humanmedizin an der Universität Tübingen

6/1991 Erlangen des Doktorgrades der Medizin, Universität Tübingen, Thema: "Einfluß von Insulin, Proinsulin, IGF-I und Protirelin auf das Wachstum und die Iodidaufnahme kultivierter Schweinethyreozyten". Gesamturteil: sehr gut.

Berufstätigkeiten

6/1991 - 8/1992 Tätigkeit als Arzt im Praktikum an der Medizinischen Universitätsklinik Freiburg, Abteilung für Hämatologie und Onkologie, Forschungsschwerpunkt: Immunmodulatorische Effekte von IL-2 in vivo (BMFT gefördertes Projekt), Immuntherapie mit rekombinanten Zytokinen (IL-2, IL-1)

9/1992 - 1/1995 Tätigkeit als wissenschaftlicher Assistent an der Medizinischen Universitätsklinik Heidelberg, Abteilung für Hämatologie, Onkologie und Rheumatologie, Forschungsschwerpunkt: Immuntherapien von Patienten mit malignem Melanom (Zytokine, Peptide), Detektion von zirkulierenden Tumorzellen im peripheren Blut mittels RT-PCR (Deutsche Krebshilfe gefördertes Projekt)

seit 1/1997 Tätigkeit als wissenschaftlicher Assistent an der Medizinischen Universitätsklinik Tübingen, Abteilung für Hämatologie, Onkologie, Immunologie und Rheumatologie, Forschungsschwerpunkt: Entwicklung von Immuntherapien mit Antigen beladenen dendritischen Zellen (DC), Entwicklung

	und Funktion DC, Interaktion zwischen DC und T-Lymphozyten, Identifizierung neuer Tumorantigene (seit 1997 Mitglied des SFB 510, Förderung durch Deutsche Krebshilfe sowie das *fortüne*- Programm der Universität Tübingen)
seit 12/1999	**Facharzt für Innere Medizin**
seit 6/2000	Vertreter der Medizinischen Klinik bei der interdisziplinären Konferenz für Hauttumore
13.02.2001	**Habilitation für das Fach Innere Medizin**
Seit Februar 2001	**Klinischer Oberarzt**, Medizinische Universitätsklinik Tübingen
Seit 1/2001	Leiter der Abteilung für Experimentelle Immuntherapien

Auslandsaufenthalt

2/1995 - 1/1997	Forschungsaufenthalt als Stipendiat der Deutschen Krebshilfe am Howard-Hughes-Medical-Institute, Department of immunology, University of Washington, Seattle, USA (Dr. M. J. Bevan)

Preise

6/1993	Posterpreis, Third International Symposium on Cytokines in Hematology, Oncology, and Immunology in Hannover, June 23, 1993
10/1993	Posterpreis, verliehen von der Deutschen Gesellschaft für Hämatologie und Onkologie
10/1998	**Arthur-Pappenheim Preis** für Hämatologie und hämatologische Onkologie, verliehen von der Deutschen Gesellschaft für Hämatologie und Onkologie
11/2000	**Best Abstract Award**, verliehen von der Deutschen Gesellschaft für Hämatologie und Onkologie
12/2000	Award der American Society of Hematology

List of Publications

a) Scientific Papers

1. Wahl R, Brossart P, Eizenberger D, Schuch H, Kallee E. Direct effects of protirelin (TRH) on cultured porcine thyrocytes. **J Endocrinol Invest** 15: 345-51, 1992.

2. Lindemann A, Brossart P, Höffken K, Flaßhove M, Voliotis D, Diehl V, Hecker G, Wagner H, Mertelsmann R. Immunomodulatory effects of ultra-low-dose interleukin-2 in cancer patients: a phase IB study. **Cancer Immunol Immunother** 37: 307-15, 1993.

3. Brossart P, Keilholz U, Willhauck M, Scheibenbogen C, Möhler Th, Hunstein W. Hematogenous spread of malignant melanoma cells in different stages of disease. **J Invest Dermatol** 101: 887-89, 1993.

4. Scheibenbogen C, Keilholz U, Möhler T, Brossart P, Hunstein W. Phase II study of intermittent low-dose IFN-gamma in metastatic renal cell cancer. **Onkologie** 16: 461-64, 1993

5. Lindemann A, Brossart P, Höffken K, Flaßhove M, Voliotis D, Diehl V, Wagner H, Mertelsmann R. Serum cytokine levels in cancer patients treated with different schedules of ultra-low-dose interleukin-2. **J Immunother** 15: 225-30, 1994.

6. Brossart P, Keilholz U, Scheibenbogen C, Möhler Th, Willhauck M, Werner Hunstein. Detection of residual tumor cells in patients with malignant melanoma responding to immunotherapy. **J Immunother** 15: 38-41, 1994.

7. Keilholz U, Scheibenbogen C, Brossart P, Mohler T, Tilgen W, Hunstein W. Interleukin-2-based immunotherapy and chemoimmunotherapy in metastatic melanoma. **Recent Results Cancer Res** 139: 383-90, 1995.

8. Keilholz U, Scheibenbogen C, Mohler T, Brossart P, Maclachlan D, Geueke AM, Jochim A, Hunstein W. Addition of dacarbazine or cisplatin to interferonalpha/ interleukin-2 in metastatic melanoma: toxicity and immunological effects. **Melanoma Res** 5: 283-7, 1995.

9. Mohler T, Willhauck M, Scheibenbogen C, Pawlita M, Bludau H, Brossart P, Hunstein W, Keilholz-U. Restriction of T cell receptor V beta repertoire in melanoma metastasis responding to immunotherapy. **Melanoma Res** 5: 129-32, 1995.

10. Willhauck M, Möhler T, Scheibenbogen C, Pawlita M, Brossart P, Schmier JW, Keilholz-U. T-cell receptor beta variable region diversity in melanoma metastases after interleukin 2-based immunotherapy. **Clin Cancer Res** 2: 767-772, 1996.

11. Brossart P, Schmier JW, Kruger S, Willhauck M, Scheibenbogen C, Mohler T, Keilholz U. A polymerase chain reaction-based semiquantitative assessment of malignant melanoma cells in peripheral blood. **Cancer Res** 55: 4065-68, 1995.

12. Brossart P, Bevan MJ. Selective activation of Fas/Fas ligand-mediated cytotoxicity by a self peptide. **J Exp Med** 183: 2449-58, 1996.

13. Brossart P, Goldrath AW, Butz EA, Martin S, Bevan MJ. Adenovirus mediated delivery of antigenic epitopes into DC by a means of CTL induction. **J Immunol** 158:3270-76, 1997.

14. Brossart P, Bevan MJ. Presentation of exogenous antigens on MHC class I molecules by dendritic cells: pathway of presentation and regulation by cytokines. **Blood** 90: 1594-99, 1997.

15. Brossart P, Stuhler G, Flad T, Stevanovic S, Rammensee H.-G, Kanz L, Brugger W. Her-2/neu derived peptides are tumor-associated antigens expressed by human renal cell and colon carcinoma lines and are recognized by in vitro induced specific cytotoxic T lymphocytes. **Cancer Res** 58: 732-736, 1998.

16. Brossart P, Grünebach F, Stuhler G, Reichardt V, Möhle R, Kanz L, Brugger W. Generation of functional human dendritic cells from adherent peripheral blood mononuclear cells by CD40 ligation in the absence of GM-CSF. **Blood** 92: 4328-47, 1998.

17. Flad T, Spengler B, Kalbacher H, Brossart P, Baier DM, Kaufmann R, Bold P, Metzger S, Meyer H, Kurz B, Müller CA. Direct identification of MHC Class I-bound tumor-associated peptide antigens of a renal carcinoma cell line by a novel mass spectrometric method. **Cancer Res** 58: 55803, 1999.

18. Stuhler G, Zobiwalski A, Grünebach F, Brossart P, Reichardt VL, Barth, H, Stevanovic S, Brugger W, Kanz L, Schlossmann SF. Immune regulatory loops determine productive interactions within human T lymphocyte-dendritic cell clusters. **PNAS** 96: 1532, 1999.

19. Brossart P, Heinrich KS, Stuhler G, Behnke L, Reichardt VL, Stevanovic S, Muhm A, Rammensee HG, Kanz L, Brugger W: Identification of HLA-A2-restricted T-cell epitopes derived from the **MUC1** tumor antigen for broadly applicable vaccine therapies. **Blood** 93:4309, 1999.

20. Brugger W, Buhring HJ, Grunebach F, Vogel W, Kaul S, Muller R, Brummendorf TH, Ziegler BL, Rappold I, Brossart P, Scheding S, Kanz L Expression of MUC-1 epitopes on normal bone marrow: implications for the detection of micrometastatic tumor cells. **J Clin Oncol** 17:1535-44, 1999.

21. Brugger W, Brossart P, Scheding S, Stuhler G, Heinrich K, Reichardt V, Grunebach F, Buhring HJ, Kanz L. Approaches to dendritic cell-based immunotherapy after peripheral blood stem cell transplantation. **Ann N Y Acad Sci** 872:363-71, 1999.

22. Brossart P, Spahlinger B, Grünebach F, Stuhler G, Reichardt VL, Kanz L, Brugger W. Induction of minor histocompatibility antigen HA-1 specific cytotoxic Tcells for the treatment of leukemia after allogeneic stem cell transplantation. (research letter) **Blood** 94: 4374-76, 1999

23. Riegler S, Hebart H, Einsele H, Brossart P, Jahn G , Sinzger C. Monocytederived dendritic cells are permissive to the complete replicative cycle of human cytomegalovirus. **J Gen Virol** 81: 393-399, 2000

24. Kugler A, Stuhler G, Walden P, Zöller G, Zobywalski A, Brossart P, Trefzer U, Ullrich S, Müller CA, Becker V, Gross AJ, Hemmerlein B, Kanz L, Müller GA, Ringert RH. Regression of human metastatic renal cell carcinoma after vaccination with tumor cell-dendritic cell hybrids. **Nature Med** 3: 332-336, 2000

25. Brossart P, Zobywalski A, Grünebach F, Behnke L, Stuhler G, Reichardt VL, Kanz L, Brugger W. TNF-α and CD40 ligand antagonize the inhibitory effects of IL-10 on T-cell stimulatory capacity of dendritic cells. **Cancer Res** 60:4485-92, 2000

26. Brossart P, Wirths S, Stuhler G, Reichardt VL, Kanz L, Brugger W. Induction of CTL responses in vivo after vaccinations with peptide pulsed dendritic cells. **Blood** 96:3102-8, 2000.

27. Seifert M, Brossart P, Cant C, Cella M, Colonna M, Brugger W, Kanz L, Ulrich A, Bühring HJ. Signal-regulatory protein (SIRP)α but not SIRPß is involved in T-cell activation, binds to CD47 with high affinity, and is expressed on immature CD34+CD38- hematopoetic cells, **Blood**, 97:2741-9, 2001

28. Kleihauer A, Grigoleit U, Hebart H, Brossart P, Alexandra Muhm A, Stevanovic S, Rammensee HG, Sinzger C, Riegler S, Jahn G, Kanz L, H Einsele. Ex-vivo generation of HCMV-specific cytotoxic T Cells by peptidepulsed de ndritic cells, **British Journal of Hematology**, 113: 231-9, 2001

29. Brossart, P, Wirths, S, Brugger, W, Kanz, L. Dendritic cells in cancer vaccines. **Exp. Hematology**, in press

30. Nencioni, A and Brossart, P. New perspectives in dendritic cells-based cancer immunotherapy. **BioDrugs**, in press

31. Brugger W, Schneider, A, Dill, P, Schammann, T, Grünebach, F Buhring HJ, Kanz L, Brossart P. Dendritic cell based vaccines in patients with haematological malignancies . **Ann N Y Acad Sci** , 938:359-62, 2001

32. Brossart, P, Schneider, A, Dill, P, Schammann, T, Grünebach, F, Wirths, S, Lothar Kanz, L, Bühring, HJ, Brugger, W. The epithelial mucin MUC-1 is expressed in hematological malignancies and is recognized by MUC1 specific cytotoxic Tlymphocytes, **Cancer Res.**, in press.

33. Wirths, S, Reichert, J, Grünebach, F, Brossart, P. Activated CD8 positive Tlymphocytes restore the stimulatory capacity of IL-10 treated antigen presenting cells, submitted.

Publikationsverzeichnis

a) Wissenschaftliche Arbeiten

1. Wahl R, Brossart P, Eizenberger D, Schuch H, Kallee E. Direct effects of protirelin (TRH) on cultured porcine thyrocytes. **J Endocrinol Invest** 15: 345-51, 1992.

2. Lindemann A, Brossart P, Höffken K, Flaßhove M, Voliotis D, Diehl V, Hecker G, Wagner H, Mertelsmann R. Immunomodulatory effects of ultra-low-dose interleukin-2 in cancer patients: a phase IB study. **Cancer Immunol Immunother** 37: 307-15, 1993.

3. Brossart P, Keilholz U, Willhauck M, Scheibenbogen C, Möhler Th, Hunstein W. Hematogenous spread of malignant melanoma cells in different stages of disease. **J Invest Dermatol** 101: 887-89, 1993.

4. Scheibenbogen C, Keilholz U, Möhler T, Brossart P, Hunstein W. Phase II study of intermittent low-dose IFN-gamma in metastatic renal cell cancer. **Onkologie** 16: 461-64, 1993

5. Lindemann A, Brossart P, Höffken K, Flaßhove M, Voliotis D, Diehl V, Wagner H, Mertelsmann R. Serum cytokine levels in cancer patients treated with different schedules of ultra-low-dose interleukin-2. **J Immunother** 15: 225-30, 1994.

6. Brossart P, Keilholz U, Scheibenbogen C, Möhler Th, Willhauck M, Werner Hunstein. Detection of residual tumor cells in patients with malignant melanoma responding to immunotherapy. **J Immunother** 15: 38-41, 1994.

7. Keilholz U, Scheibenbogen C, Brossart P, Mohler T, Tilgen W, Hunstein W. Interleukin-2-based immunotherapy and chemoimmunotherapy in metastatic melanoma. **Recent Results Cancer Res** 139: 383-90, 1995.

8. Keilholz U, Scheibenbogen C, Mohler T, Brossart P, Maclachlan D, Geueke AM, Jochim A, Hunstein W. Addition of dacarbazine or cisplatin to interferon-alpha/interleukin-2 in metastatic melanoma: toxicity and immunological effects. **Melanoma Res** 5: 283-7, 1995.

9. Mohler T, Willhauck M, Scheibenbogen C, Pawlita M, Bludau H, Brossart P, Hunstein W, Keilholz-U. Restriction of T cell receptor V beta repertoire in melanoma metastasis responding to immunotherapy. **Melanoma Res** 5: 129-32, 1995.

10. Willhauck M, Möhler T, Scheibenbogen C, Pawlita M, Brossart P, Schmier JW, Keilholz-U. T-cell receptor beta variable region diversity in melanoma metastases after interleukin 2-based immunotherapy. **Clin Cancer Res** 2: 767-772, 1996.

11. Brossart P, Schmier JW, Kruger S, Willhauck M, Scheibenbogen C, Mohler T, Keilholz U. A polymerase chain reaction-based semiquantitative assessment of malignant melanoma cells in peripheral blood. **Cancer Res** 55: 4065-68, 1995.

12. Brossart P, Bevan MJ. Selective activation of Fas/Fas ligand-mediated cytotoxicity by a self peptide. **J Exp Med** 183: 2449-58, 1996.

13. Brossart P, Goldrath AW, Butz EA, Martin S, Bevan MJ. Adenovirus mediated delivery of antigenic epitopes into DC by a means of CTL induction. **J Immunol** 158:3270-76, 1997.

14. Brossart P, Bevan MJ. Presentation of exogenous antigens on MHC class I molecules by dendritic cells: pathway of presentation and regulation by cytokines. **Blood** 90: 1594-99, 1997.

15. Brossart P, Stuhler G, Flad T, Stevanovic S, Rammensee H.-G, Kanz L, Brugger W. Her-2/neu derived peptides are tumor-associated antigens expressed by human renal cell and colon carcinoma lines and are recognized by in vitro induced specific cytotoxic T lymphocytes. **Cancer Res** 58: 732-736, 1998.

16. Brossart P, Grünebach F, Stuhler G, Reichardt V, Möhle R, Kanz L, Brugger W. Generation of functional human dendritic cells from adherent peripheral blood mononuclear cells by CD40 ligation in the absence of GM-CSF. **Blood** 92: 4328-47, 1998.

17. Flad T, Spengler B, Kalbacher H, Brossart P, Baier DM, Kaufmann R, Bold P, Metzger S, Meyer H, Kurz B, Müller CA. Direct identification of MHC Class I-bound tumor-associated peptide antigens of a renal carcinoma cell line by a novel mass spectrometric method. **Cancer Res** 58: 55803, 1999.

18. Stuhler G, Zobiwalski A, Grünebach F, Brossart P, Reichardt VL, Barth, H, Stevanovic S, Brugger W, Kanz L, Schlossmann SF. Immune regulatory loops determine productive interactions within human T lymphocyte-dendritic cell clusters. **PNAS** 96: 1532, 1999.

19. Brossart P, Heinrich KS, Stuhler G, Behnke L, Reichardt VL, Stevanovic S, Muhm A, Rammensee HG, Kanz L, Brugger W: Identification of HLA-A2-restricted T-cell epitopes derived from the MUC1 tumor antigen for broadly applicable vaccine therapies. **Blood** 93:4309, 1999.

20. Brugger W, Buhring HJ, Grunebach F, Vogel W, Kaul S, Muller R, Brummendorf TH, Ziegler BL, Rappold I, Brossart P, Scheding S, Kanz L Expression of MUC-1 epitopes on normal bone marrow: implications for the detection of micrometastatic tumor cells. **J Clin Oncol** 17:1535-44, 1999.

21. Brugger W, Brossart P, Scheding S, Stuhler G, Heinrich K, Reichardt V, Grunebach F, Buhring HJ, Kanz L. Approaches to dendritic cell-based immunotherapy after peripheral blood stem cell transplantation. **Ann N Y Acad Sci** 872:363-71, 1999.

22. Brossart P, Spahlinger B, Grünebach F, Stuhler G, Reichardt VL, Kanz L, Brugger W. Induction of minor histocompatibility antigen HA-1 specific cytotoxic T-cells for the treatment of leukemia after allogeneic stem cell transplantation. (research letter) **Blood** 94: 4374-76, 1999

23. Riegler S, Hebart H, Einsele H, Brossart P, Jahn G , Sinzger C. Monocyte-derived dendritic cells are permissive to the complete replicative cycle of human cytomegalovirus. **J Gen Virol** 81: 393-399, 2000

24. Kugler A, Stuhler G, Walden P, Zöller G, Zobywalski A, Brossart P, Trefzer U, Ullrich S, Müller CA, Becker V, Gross AJ, Hemmerlein B, Känz L, Müller GA, Ringert RH. Regression of human metastatic renal cell carcinoma after vaccination with tumor cell-dendritic cell hybrids. **Nature Med** 3: 332-336, 2000

25. Brossart P, Zobywalski A, Grünebach F, Behnke L, Stuhler G, Reichardt VL, Kanz L, Brugger W. TNF-α and CD40 ligand antagonize the inhibitory effects of IL-10 on T-cell stimulatory capacity of dendritic cells. **Cancer Res** 60:4485-92, 2000

26. Brossart P, Wirths S, Stuhler G, Reichardt VL, Kanz L, Brugger W. Induction of CTL responses in vivo after vaccinations with peptide pulsed dendritic cells. **Blood** 96:3102-8, 2000.

27. Seifert M, Brossart P, Cant C, Cella M, Colonna M, Brugger W, Kanz L, Ulrich A, Bühring HJ. Signal-regulatory protein (SIRP)α but not SIRPß is involved in T-cell activation, binds to CD47 with high affinity, and is expressed on immature CD34+CD38-hematopoetic cells, **Blood**, 97:2741-9, 2001

28. Kleihauer A, Grigoleit U, Hebart H, Brossart P, Alexandra Muhm A, Stevanovic S, Rammensee HG, Sinzger C, Riegler S, Jahn G, Kanz L, H Einsele. Ex-vivo generation of HCMV-specific cytotoxic T Cells by peptide-pulsed dendritic cells, **British Journal of Hematology**, 113: 231-9, 2001

29. Brossart, P, Wirths, S, Brugger, W, Kanz, L. Dendritic cells in cancer vaccines. **Exp. Hematology**, in press

30. Nencioni, A and Brossart, P. New perspectives in dendritic cells-based cancer immunotherapy. **BioDrugs**, in press

31. Brugger W, Schneider, A, Dill, P, Schammann, T, Grünebach, F Buhring HJ, Kanz L, Brossart P. Dendritic cell based vaccines in patients with hematological malignancies . **Ann N Y Acad Sci** , 938:359-62, 2001

32. Brossart, P, Schneider, A, Dill, P, Schammann, T, Grünebach, F, Wirths, S, Lothar Kanz, L, Bühring, HJ, Brugger, W. The epithelial mucin MUC-1 is expressed in hematological malignancies and is recognized by MUC1 specific cytotoxic T-lymphocytes, **Cancer Res.**, in press.

33. Wirths, S, Reichert, J, Grünebach, F, Brossart, P. Activated CD8 positive T-lymphocytes restore the stimulatory capacity of IL-10 treated antigen presenting cells, submitted.